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PATHOGENIC BACTERIA IN HOG CHOLERA BLOOD

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A great deal of interest is centered about the question of the importance of pathogenic secondary invading bacteria in hog cholera blood. This question is of practical interest on account of the possible causal relationship of these secondary invaders to the losses which frequently occur in hogs injected with large amounts of hog cholera blood in the production of antihog cholera serum. In this study it is attempted to determine the relationship between the presence of gas-producing bacteria in hog cholera blood and the pathogenicity of such blood for rabbits and hogs.

Gas Production and Pathogenicity Tests.—One c c of each sample of hog cholera blood was inoculated into fermentation tubes of pork infusion broth containing 1% of glucose. The inoculated tubes were then incubated for 3 days, with observation on each day for gas production. Rabbits were inoculated subcutaneously with 1 to 2 c c of cholera blood. Hogs were injected intravenously with 4 to 6 c c of hog cholera blood per pound body weight.

The hog cholera blood was obtained from cholera-susceptible pigs 5 to 9 days after the pigs had been injected with hog cholera blood for the purpose of propagating the hog cholera virus. Table 1 shows the percentage of gas-producing samples obtained by bleeding on different days after inoculation.

The inoculation of 15 rabbits with gas-producing samples of hog cholera blood resulted in the death of 10, while of 51 rabbits inoculated with nongas-producing blood only one died. With one exception, the deaths of these rabbits occurred 7 to 12 days subsequent to inoculation. This single exception occurred in the case of the one rabbit that died following inoculation with nongas-producing blood. This rabbit died on the third day after inoculation, and an organism morphologically and culturally B. suisepticus-like was isolated from the heart blood.

The data in table 1 indicate that, after the fifth day following inoculation of hogs with cholera virus, there was a marked increase in the proportion of cholera-infected hogs having gas-producing bacteria in their blood. This proportion was much higher on the eighth and ninth days than on the sixth and seventh days. The results of inoculations of rabbits show a close relationship between the presence of gas-producing organisms in hog cholera blood and the pathogenicity of such

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blood for rabbits. It appeared that, with one exception, hog cholera blood which did not contain organisms capable of fermenting glucose with the production of gas was harmless for rabbits when inoculated subcutaneously. This single exception, in 51 cases, was one in which a B. suisepticus-like organism was isolated from the heart blood of a rabbit that died 3 days after inoculation with nongas-producing cholera blood.

TABLE 1

RELATION OF PERCENTAGE OF GAS-PRODUCING SAMPLES OF HOG CHOLERA BLOOD TO TIME OF BLEEDING

Samples	Day of Bleeding	Percentage Producing Gas
20	5-day	0.0
79	6-day	6.7
162	7-day	6.8
14	8-day	14.2
15	9-day	13.3
566	Unclassified*	12.0

* This group consisted of 7, 8 and 9-day virus.

While the hog cholera blood containing gas-producing organisms appears to be virulent to a high degree for rabbits, the same blood may be harmless for hogs, even when injected intravenously in large quantities. Thus the common assumption of correlation of the pathogenicity of such organisms for rabbits and swine does not appear to be sustained. Of 72 hogs injected with cholera blood containing gas-producing bacteria, only one died; whereas, of 175 hogs injected with cholera blood which did not contain gas-producing bacteria, 8 died. While these hogs had previously been vaccinated and were presumably immune, it is quite possible that they did not possess a degree of immunity sufficient to withstand the large dose of hog cholera virus. Susceptibility in presumably immune hogs has been demonstrated on other occasions, and, granting such susceptibility in the presence of the filterable hog cholera virus, the presence or absence of bacteria probably has no material influence on the course of the disease.

Bacteriologic Examination.—Since the results of rabbit inoculation with hog cholera blood indicated a close relationship between the presence of gas-producing bacteria and pathogenicity of such blood for rabbits, a qualitative bacteriologic examination was made of a number of samples of cholera blood. As this study has been reported in part in a previous publication,[1] only a brief outline of the technic employed will be given.

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[1] Spray, R. S.: Jour. Infec. Dis., 1920, 26, p. 340.

Cultures were isolated by dilution plating from glucose broth fermentation tubes or by direct plating of the hog cholera blood. Cultures were isolated from rabbits by plating heart blood of animals dying following inoculation with hog cholera blood. These cultures were replated several times to insure purity, and stock cultures were picked from typical well isolated colonies. Preliminary and presumptive paratyphoid tests were made on these stock cultures, and cultures not conforming to the characteristics of the paratyphoid-enteritidis group were discarded. Having satisfied the presumptive tests, the cultures were put through a series of cultural tests, the results of which are recorded in table 2.

TABLE 2

ESSENTIAL DIAGNOSTIC CHARACTERISTICS

Cultures	Glucose Serum Water	Lead Acetate Agar	Milk Alkaline at	Translucent Opalescent at 90 Days	Arabinose Serum Water	Xylose Serum Water	Dulcite Serum Water	Inosite Serum Water
B. suipestifer type Strain 49..	+	—	4th day	+	— —	++	— —	— —
Virus 108, 125, 127, 138, 145, 146, 161, 167, 187, 192, 193.....	+	—	8-10 days	+	— —	++	— —	— —
Rabbit 107*..................	+	—	8th day	+	— —	++	— —	— —
B. paratyphosus A, Human A98........................	—	+	38th day	—	+ —	— —	++	— —
Human A97, Rabbit 134*, Virus 149....................	+	—	35-38 days	—	+ —	— —	++	— —
B. paratyphosus B, Human B96, Rabbit 124, 135*........	—	+	3-4 days	+	++	++	++	++
Intermediate, Rabbit 91*, Virus 201, 202...............	—	+	6-7 days	+	— —	++	— —	— —
B. enteritidis, Human E117...	—	+	2d day	+	++	++	++	— —

* Strains isolated from rabbits inoculated with virus blood known to contain gas-producing bacteria.

Table 3 indicates that, while 12 strains of typical B. suipestifer were isolated, other paratyphoids may frequently be encountered in the study of hog cholera blood. Two strains each of organisms identical with B. paratyphosus A and B strains of human origin and three interesting intermediates were isolated from cholera blood or from rabbits dying after inoculation with cholera blood. In the study of another disease of swine [1] on two occasions intermediate strains, identical with those isolated from cholera blood and rabbits, were also isolated from the spleen. The significance of the presence, and the relationships of these intermediates are uncertain, but they apparently constitute a well-defined and constant type which may be frequently encountered in the study of pathologic swine tissue.

In order to compare and study these intermediates further, cross-agglutination tests were made with authentic strains of B. paratyphosus A and B, B. enteritidis, and B. suipestifer.

TABLE 3

HIGHEST SERUM DILUTION SHOWING PERCEPTIBLE AGGLUTINATION

Cultures	Serum				
	B. suipestifer 49	Intermediate 91	B. paratyphosus B96	B. paratyphosus A98	B. enteritidis E117
B. suipestifer type, Strain 49	1:30000	1:15000	1:3000	....	....
Virus 108, 125, 127, 138, 145, 146, 161, 167, 187, 192, 193	1:30000	1:15000	1:3000	....	....
Rabbit 107	1:30000	1:15000	1:3000	....	....
B. paratyphosus A. Human A98	....	....	....	1:15000	....
Human A97, Rabbit 134, Virus 149	....	....	....	1:15000	....
B. paratyphosus B, Human A96, Rabbit 124, 135	1:3000	1:3000	1:15000	....	....
Intermediate, Rabbit 91, Virus 201, 202	1:15000	1:30000	1:7500	....	....
B. enteritidis, Human E117	....	....	....	....	1:7500

TABLE 4

ABSORPTION TESTS SHOWING RELATIONSHIP BETWEEN INTERMEDIATES, B. SUIPESTIFER AND B. PARATYPHOSUS B

Cultures	Serums								
	B. suipestifer 49			Intermediate 91			B. paratyphosus B96		
	Absorbed by Intermediate 91	Absorbed by B. paratyphosus B 135	Absorbed by B. suipestifer 107	Absorbed by B. suipestifer 49	Absorbed by B. paratyphosus B 135	Absorbed by Intermediate 201	Absorbed by B. suipestifer 49	Absorbed by Intermediate 91	Absorbed by B. paratyphosus B 135
B. suipestifer Type 49, 11 virus strains, Rabbit 107	1:30000	1:30000	....	1:750	1:15000	....	....	....	....
B. paratyphosus B, Human B96, Rabbit 124, 135	....	....	....	....	....	....	1:15000	1:15000	....
Intermediate, Rabbit 91 Virus 201, 202	....	1:15000	....	1:7500	1:30000	....	....	....	....

The results indicate a close relationship between the intermediate strains and typical B. suipestifer, and to a lesser degree with B. paratyphosus B. So close was the agglutinative affinity between the intermediate strains and B. suipestifer that, with liberal allowance for cultural variation, these strains might be regarded as aberrant strains of B. suipestifer. As noted,[1] the cultural evidence of the identity of strains 134, 149, and 124, 135 with human strain of B. paratyphosus A and B, respectively, is also supported by these agglutination tests. These strains were further studied by absorption of agglutinins (table 4).

It is immediately apparent that the three intermediate strains constitute a distinct type, incapable of absorbing specific agglutinins from B. suipestifer antiserum. Peculiarly, however, both B. suipestifer and B. paratyphosus B can, to a certain extent, absorb agglutinins from an antiserum against the intermediate strains.

SUMMARY

After the fifth day following inoculation with hog cholera virus there was a marked increase in the proportion of cholera infected hogs having gas-producing bacteria in the blood.

Hog cholera blood containing gas-producing bacteria was usually fatal to rabbits when injected subcutaneously.

Hog cholera blood not containing gas-producing bacteria was nearly uniformly harmless to rabbits when injected subcutaneously.

Hog cholera blood containing gas-producing bacteria was no more harmful than blood not containing gas-producing bacteria when injected in large quantities intravenously into hogs in the production of antihog cholera serum.

One strain of B. paratyphosus A was isolated by direct plating of hog cholera blood; another strain of B. paratyphosus A, and two strains of B. paratyphosus B, were isolated from the heart blood of rabbits dying following inoculation with hog cholera blood. These strains were identical in all respect with human strains of B. paratyphosus A and B.

Three identical intermediates were isolated, two by direct plating of hog cholera blood, and one from heart blood of a rabbit which died after inoculation with hog cholera blood. This same type has been isolated before and since from the spleens of pigs.